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FERMENTABILITY TESTS OF SUGARS PRODUCED IN THE SOLAR ENERGY RESEARCH INSTITUTE'S PLUG-FLOW REACTOR

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ABSTRACT

An experiment was designed to determine the relative fermentability of hydrolyzates produced in the Solar Energy Research Institute's (SERI) plug-flow reactor.

Two hydrolyzates were prepared for fermentability testing by raising the pH to 5.5 with calcium oxide (CaO) or overneutralizing with CaO, filtering, and adding sulfuric acid (H_2SO_4) to reach pH 5.5. Prepared hydrolyzates were tested at full, half, and quarter strength. Test solutions were inoculated with Saccharomyces cerevisiae, S. uvarum, or a coculture of Candida lusitaniae and Candida sp.

Half- and quarter-strength hydrolyzates proved fermentable by the individual yeasts and the coculture without regard for the type of preparation for fermentability testing. Conversion efficiencies were 0.45 g ethanol/g glucose or better.

Full-strength hydrolyzates were not fermented using S. uvarum. Overneutralization greatly improved fermentation of full-strength hydrolyzates using S. cerevisiae and the coculture. A slightly better ethanol yield was achieved in the hydrolyzate with the greater amount of glucose.

Fermentability test results have implications for means to recover near-theoretical yields of ethanol from hydrolyzates produced in the plug-flow reactor.

FERMENTABILITY TESTS OF SUGARS PRODUCED IN THE SOLAR ENERGY RESEARCH INSTITUTE'S PLUG-FLOW REACTOR

INTRODUCTION

The purpose of this work is to determine the fermentability to ethanol of cellulose-derived sugars produced in the Solar Energy Research Institute's (SERI) plug-flow reactor. The hydrolyzates were treated for fermentation by two methods. The solutions were tested at full, half, and quarter strength. Fermentability by two glucose-fermenting yeasts and a coculture was tested.

MATERIALS AND METHODS

Treatment of Hydrolyzates

Two containers were received from SERI and labeled 1 and 2. Three liters of each of the solutions were weighed and divided into two equal portions. One portion of each hydrolyzate was adjusted to pH 5.5 with CaO and filtered through Whatman No. 42 paper. This portion was labeled as A. The other portion of each was overneutralized to pH 9, filtered, and adjusted to pH 5.5 with concentrated H_2SO_4. This portion was labeled as B. Each of the four portions was treated with Na_2SO_3 (0.1 w/v percent); yeast extract (0.2 w/v percent), urea (0.2 w/v percent), and KH_2PO_4 (0.05 w/v percent) were added.

Dilutions were made of the four portions so that fermentation tests could be made on duplicate full-, half-, and quarter-strength, 95-ml aliquots. These aliquots were filtered through a 0.2 μm filter for sterility.

Yeast:

Saccharomyces cerevisiae (ATCC 24860), S. uvarum (ATCC 26602), and a coculture of Candida lusitaniae (ATCC 34449) and Candida sp. (ATCC 20615) were used to inoculate the prepared sugar solutions. The yeast cultures were maintained on YM agar (Difco) slants. Inocula were prepared by growing yeasts for 24 hours on YM agar and transferring growth to 100-ml aliquots of a 2-percent (w/v) glucose plus 0.67-percent (w/v) YNB (Difco) medium. After a 48-hour incubation at 32°C and 100 r/min in a rotary shaker, the yeasts were harvested by centrifugation and used to inoculate the test aliquots at the rate of 5 percent by volume or 0.1 to 0.2 g/L yeast.

Fermentation Tests:

Test solutions (treated sugar solutions plus yeast inoculum) of 100-ml total volume were incubated at 32°C and 50 r/min in a rotary shaker. Solutions were contained in sterile, disposable, Erlenmeyer flasks with caps tightened and vented with 25-gauge needles. Solutions were sampled at 1, 2, 3, and 7 days.

Analytical Methods:

The yeast density was determined gravimetrically. Sugars and other organics were determined by high-performance liquid chromatography [Spectra-Physics 8000 equipped with Aminex HPX-85 and with HPX-87P (Bio-Rad) ion-exchange columns]. Metals were determined by argon plasma spectrophotometry of perchloric acid digests of samples.

RESULTS AND DISCUSSION

The results of adjusting the pH to 5.5 with CaO and of over-neutralization on the sugars and organics can be seen in Table 1. Data are averages of analyses of duplicate samples. There is a variability in the analyses of the sugars which makes it difficult to determine whether the treatments affected the concentrations of sugars; however, the distribution of sugars in sample 1 before and after the treatments is 75-percent glucose, 19-percent xylose, and 6-percent other. The distribution of sugars in sample 2 before and after the treatments is 70-percent glucose, 23-percent xylose, and 7-percent other.

Both treatments caused a decrease in the concentration of furfural in the sugar solutions. The fate of the metals was not followed during the treatments. The levels of these metals in the sugar solutions were not anticipated to inhibit the yeasts.

Fermentation results from tests of half- and quarter-strength sugar solutions show that the type of treatment made little difference in the performance of the yeasts in diluted hydrolyzates. The individual yeast species and the coculture performed in the same way in diluted hydrolyzates. This was not the case with full-strength solutions; however, best ethanol yields were achieved by S. cerevisiae at all solution strengths. There was little difference in the amount of ethanol produced per unit glucose consumed from diluted as opposed to full-strength hydrolyzates 1 and 2. The conversion efficiency was about 88 percent of maximum (based on glucose) or about 0.45 g ethanol was produced per g of glucose in the dilute solutions (Tables 2 and 3).

Figure 1 shows a typical time course of fermentation of sugar solution 1 diluted to one-quarter strength, using S. uvarum. More dramatic differences in response of the yeasts to the treatments are seen with full-strength sugar solutions 1 and 2. Overliming was a decidedly better treatment for enhanced ethanol production than raising the pH to 5.5 with CaO (Figures 2 and 3). More ethanol was produced from sugar solution 2

Table 1. Analyses of Sugar Solutions from SERI's Plug-Flow-Reactor Treated for Fermentability Testing

Sample Designation*	Dilution	g/L										PPM			
		Glucose	Xylose	Galactose	Arabinose	Mannose	Formic Acid	Acetic Acid	Levulinic Acid	HMF	Furfural	Fe	Cr	Cu	Ni
1A	Before pH adjustment	17.2	4.5	0.7	<0.1	0.6	1.4	3.5	1.5	1.8	2.3	39.7	4.5	<0.01	2.7
1A	Full strength	17.7	4.6	-.7	<0.1	0.7	1.4	3.7	1.6	1.5	1.8	-	-	-	-
1A	Half strength	8.6	1.7	<0.1	<0.1	0.4	0.8	2.0	0.8	0.8	0.9	-	-	-	-
1A	Quarter strength	4.4	0.8	<0.1	<0.1	<0.1	0.4	1.1	0.4	0.4	0.5	-	-	-	-
1B	Full strength	18.3	4.7	0.6	<0.1	0.6	1.4	3.7	1.3	1.6	1.4	-	-	-	-
1B	Half strength	8.0	1.6	<0.1	<0.1	0.4	0.8	2.0	0.8	0.7	0.7	-	-	-	-
1B	Quarter strength	3.8	0.7	<0.1	<0.1	0.2	0.4	1.0	0.4	0.3	0.4	-	-	-	-
2A	Before pH adjustment	18.3	6.2	0.8	<0.1	0.7	1.2	3.4	1.2	1.5	2.1	28.3	3.2	0.01	3.5
2A	Full strength	18.1	6.0	0.8	<0.1	0.8	1.0	3.4	1.1	1.2	1.5	-	-	-	-
2A	Half strength	8.7	2.4	0.1	<0.1	0.4	0.6	2.0	0.6	0.8	0.8	-	-	-	-
2A	Quarter strength	4.6	1.1	<0.1	<0.1	<0.1	0.3	1.1	0.3	0.4	0.4	-	-	-	-
2B	Full strength	18.5	6.1	0.7	<0.1	0.9	1.1	3.7	1.2	1.3	1.6	-	-	-	-
2B	Half strength	9.1	2.7	0.2	<0.1	<0.1	0.6	2.0	0.6	0.6	0.9	-	-	-	-
2B	Quarter strength	4.4	1.1	<0.1	<0.1	0.5	0.3	1.1	0.3	0.3	0.5	-	-	-	-

* A - pH adjusted to 5.5 with CaO
B - Overneutralized to pH 9
1 - Hydrolyzate 1
2 - Hydrolyzate 2

Table 2. Results of Fermentation of Sugar Solution I Using *Saccharomyces Cerevisiae*

Sample Designation*	Dilution	Day	g/L Glucose	Xylose	Mannose	Ethanol	Formic Acid	HMF	Furfural	g Ethanol/ g Glucose Used
IA	Full	0	16.8	4.4	0.7	0	1.3	1.4	1.7	-
		1	14.7	4.1	1.8	0.9	1.4	1.5	1.2	-
		2	15.7	5.4	1.8	1.0	1.5	1.6	1.3	-
		3	14.4	4.0	1.0	1.1	1.5	1.4	1.4	0.46
		7	13.6	3.6	0.8	1.0	1.7	1.4	1.1	-
	Half	0	8.2	1.6	0.4	0	0.8	0.8	0.9	-
		1	0.8	1.6	0.5	3.8	1.6	0.4	0	0.51
		2	0	1.5	0	3.3	1.9	0.2	0	-
		3	0	1.4	0	3.5	1.8	0.2	0	-
		7	0	1.4	0	3.6	1.8	0.1	0	-
	Quarter	0	4.2	0.8	0	0	0.4	0.4	0.5	-
		1	0	0.7	0	1.9	0.9	0.2	0	0.45
		2	0	0.6	0	1.7	0.9	0.2	0	-
		3	0	0.6	0	1.6	0.8	0.1	0	-
		7	0	0.5	0	1.2	0.8	0	0	-
IB	Full	0	17.4	4.5	0.6	0	1.3	1.5	1.3	-
		1	12.7	4.2	1.9	2.0	1.7	1.3	0.6	-
		2	8.7	5.7	0.8	3.2	2.9	0.8	0	-
		3	2.2	4.1	0.7	6.3	3.5	0.4	0	0.41
		7	0	3.4	0	7.0	3.8	0.2	0	-
	Half	0	7.6	1.5	0.4	0	0.8	0.7	0.7	-
		1	0.3	1.8	0	3.9	1.6	0.4	0	0.53
		2	0	1.5	0	3.4	1.7	0.3	0	-
		3	0	1.4	0	3.6	1.6	0.2	0	0.47
		7	0	1.5	0	3.6	1.7	0.1	0	-
	Quarter	0	3.6	0.7	0.2	0	0.4	0.3	0.4	-
		1	0	0.8	0	2.0	0.9	0.2	0	0.56
		2	0	0.5	0	1.6	0.7	0.2	0	-
		3	0	0.5	0	1.7	0.7	0.1	0	0.47
		7	0	0.6	0	1.6	0.7	0.1	0	-

* A - pH adjusted to 5.5 with CaO
B - Overneutralized to pH 9

Table 3. Results of Fermentation of Sugar Solution 2
Using Saccharomyces Cerevisiae

Sample Designation*	Dilution	Day	g/L Glucose	Xylose	Mannose	Ethanol	Formic Acid	HMF	Furfural	g Ethanol/ g Glucose Used
2A	Full	0	17.2	5.7	0.8	0	1.0	1.1	1.4	-
		1	14.7	5.3	1.1	1.3	1.3	1:2	0.9	-
		2	13.2	6.6	1.0	2.1	1.8	1.1	0.5	-
		3	11.0	5.7	0.9	2.9	1.9	0.9	0.2	0.47
		7	3.8	4.8	0.8	5.9	2.8	0.4	0	0.44
	Half	0	8.3	2.3	0.4	0	0.6	0.8	0.8	-
		1	0.6	2.4	0	3.9	1.4	0.3	0	0.51
		2	0	2.0	0	3.5	1.7	0.2	0	-
		3	0	2.0	0	3.7	1.6	0.1	0	-
		7	0	2.1	0	3.7	1.6	0.1	0	-
	Quarter	0	4.4	1.0	0	0	0.3	0.4	0.4	-
		1	0	1.0	0	2.2	0.8	0.2	0	0.50
		2	0	0.9	0	1.6	0.7	0.1	0	-
		3	0	0.9	0	1.5	0.7	0.1	0	-
		7	0	0.9	0	1.7	0.6	0	0	-
2B	Full	0	17.6	5.8	0.9	0	1.0	1.2	1.5	-
		1	11.3	5.2	0.7	2.0	1.6	1.0	0.4	-
		2	3.3	7.8	0.7	6.4	3.4	0.4	0	-
		3	0	5.3	0	8.9	3.3	0.2	0	0.51
		7	0	4.6	0	7.9	3.5	0.1	0	-
	Half	0	8.6	2.6	0	0	0.6	0.6	0.9	-
		1	0	2.4	0	3.8	1.5	0.3	0	-
		2	0	2.3	0	3.9	1.6	0.3	0	0.45
		3	0	2.1	0	3.4	1.4	0.1	0	-
		7	0	2.1	0	3.6	1.4	0.1	0	-
	Quarter	0	4.2	1.0	0.5	0	0.3	0.3	0.5	-
		1	0	0.9	0	1.6	0.8	0.2	0	-
		2	0	0.8	0	1.7	0.6	0.1	0	0.40
		3	0	0.8	0	1.4	0.5	0.1	0	-
		7	0	0.8	0	1.6	0.6	0	0	-

* A - pH adjusted to 5.5 with CaO
B - Overneutralized to pH 9

Figure 1. TIME COURSE OF FERMENTATION OF QUARTER STRENGTH SUGAR SOLUTION 1 USING SACCHAROMYCES UVARUM

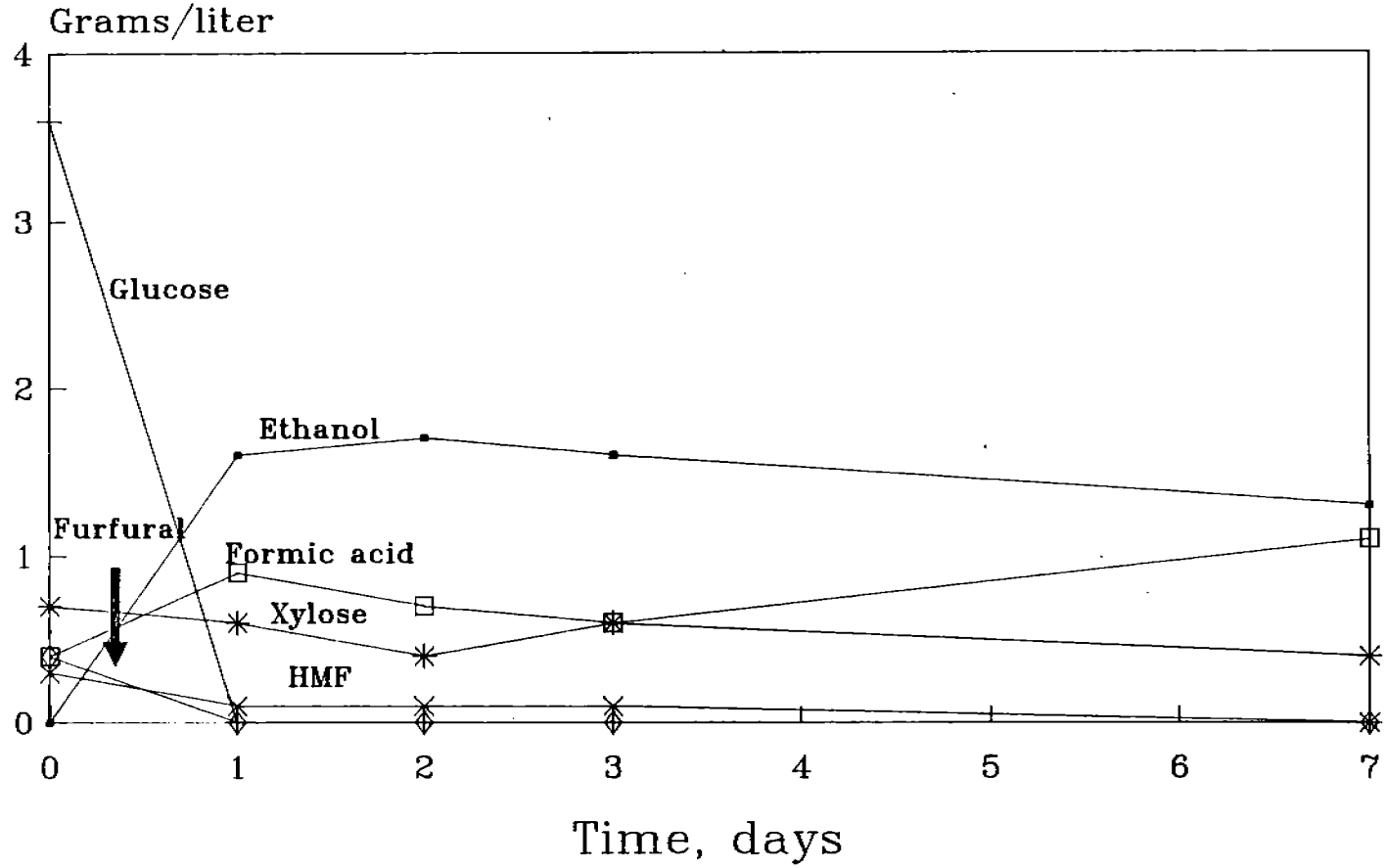
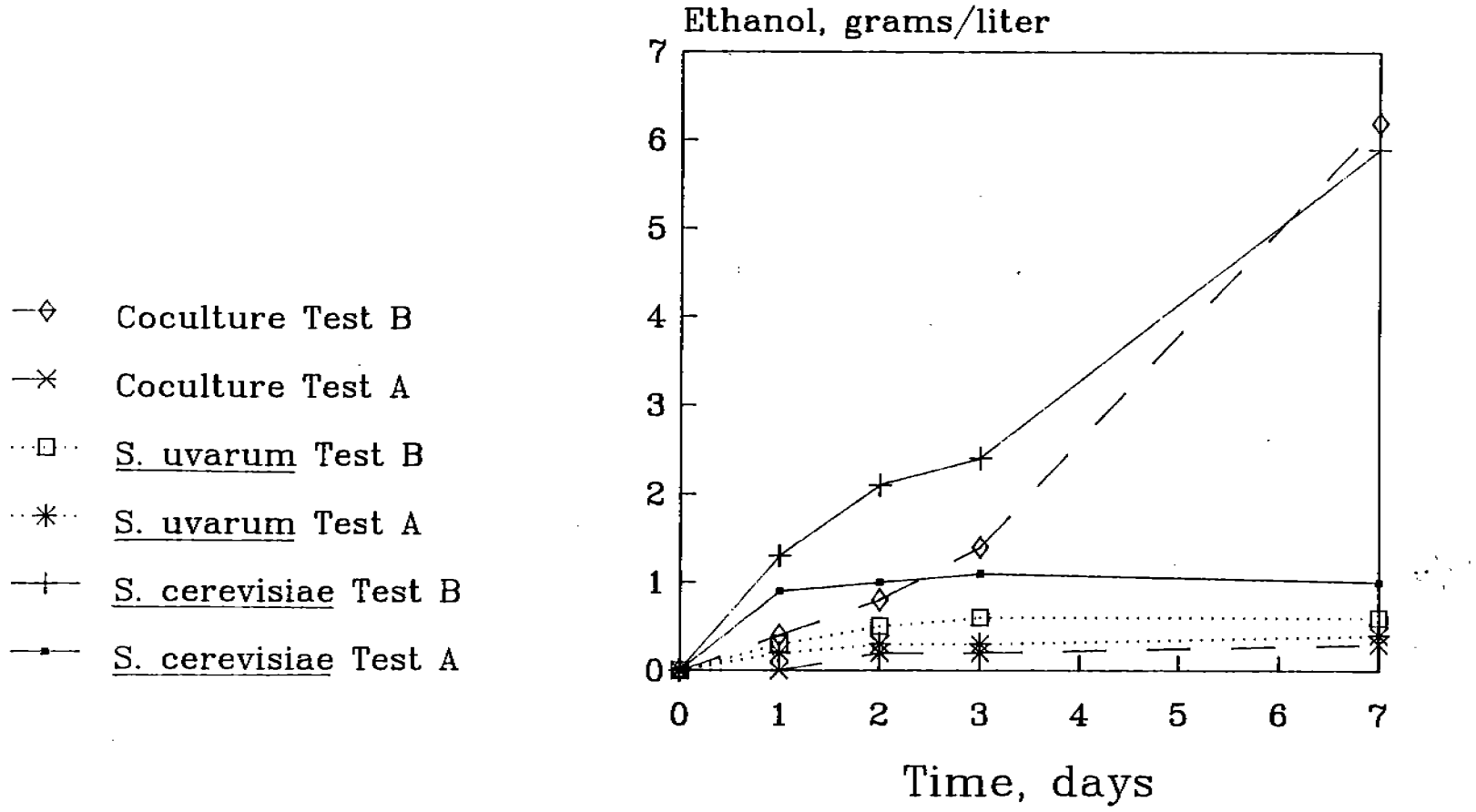


Figure 2

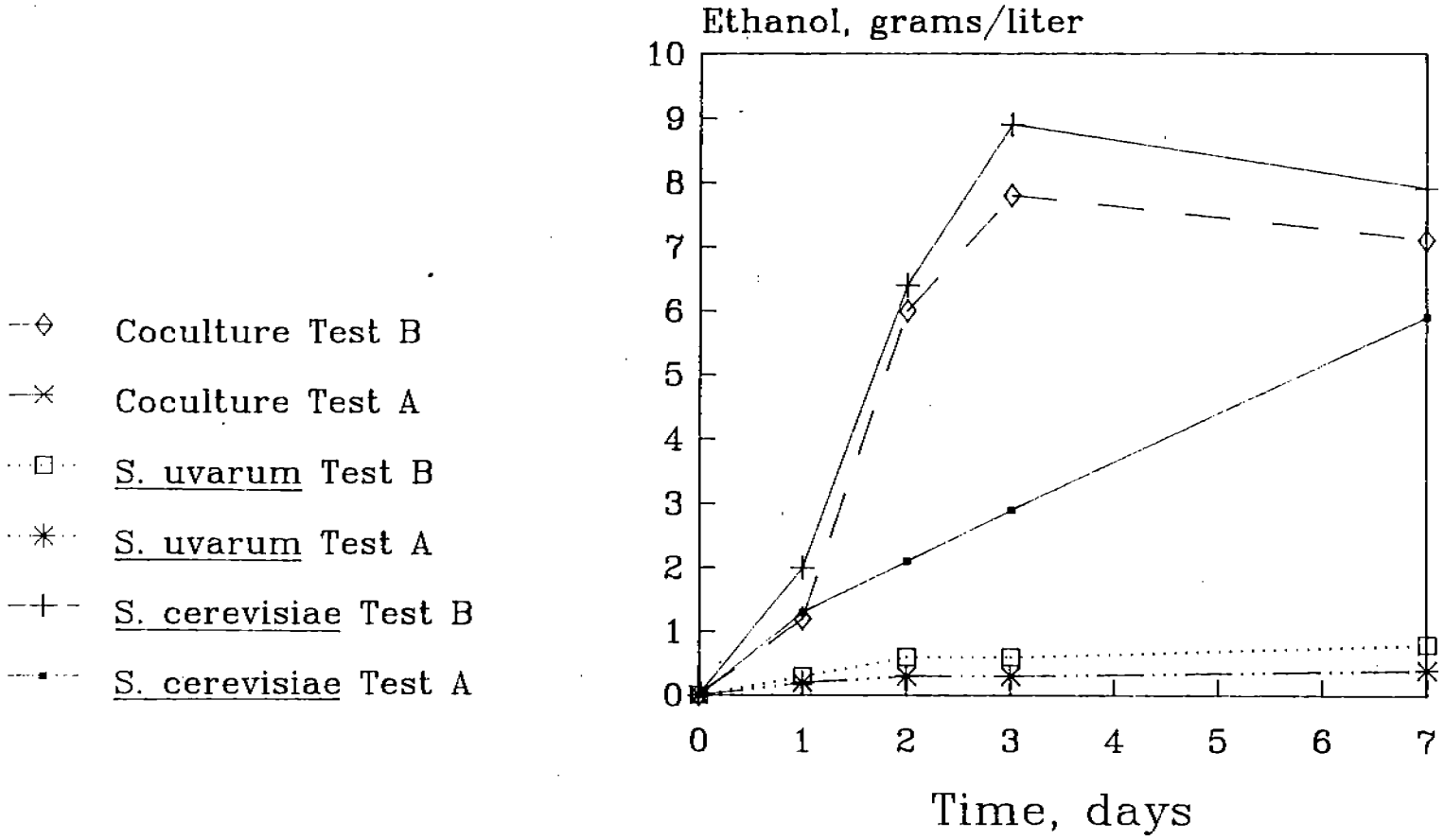
TIME COURSE OF ETHANOL PRODUCTION FROM FULL STRENGTH SUGAR SOLUTION 1 BY YEASTS



Test A – pH raised to 5.5 with CaO
Test B – overneutralized

Figure 3

TIME COURSE OF ETHANOL PRODUCTION FROM FULL STRENGTH SUGAR SOLUTION 2 BY YEASTS



Test A – pH raised to 5.5 with CaO
Test B – overneutralized

(Figure 3) than from 1. Better ethanol yields were achieved in both sugar solutions by S. cerevisiae than by S. uvarum or the coculture. The coculture performed better than S. uvarum alone.

Tables 2 and 3 include the major ethanol yield data from the fermentability tests using S. cerevisiae. The data collected for S. uvarum and the coculture are not shown since S. cerevisiae was the best performer. Analyses of xylitol, acetic acid, and levulinic acid are not shown. In some tests, a detectable level of xylitol (about 0.2 g/l) was reached at 7 days of incubation. In general, levulinic acid and acetic acid were unchanged during the course of fermentation. There is no evidence of ethanol production from xylose by S. cerevisiae nor by S. uvarum or the coculture (data not shown).

The successful fermentations of glucose to ethanol were accompanied by metabolism of furfural and hydroxymethylfurfural (HMF). The poor conversion or lack of conversion of glucose in full strength solutions to ethanol by S. uvarum and the coculture (Figures 2 and 3) was accompanied by failure to decrease the concentration of furfural and HMF (data not shown) in the sugar solution. The length of time required for ethanol to reach its greatest concentration during the course of fermentation likewise coincides with the disappearance of furfural and HMF (see Tables 2 and 3). The implications are that hydrolyzates with less of these two organics would benefit the bioconversion of the sugar solutions to ethanol by yeasts. Both raising the pH to 5.5 and overneutralizing with CaO decreased the furfural and HMF concentrations somewhat. The improved fermentation by S. cerevisiae in the overneutralized solutions may be due in part to decreased furfural and HMF and also removal of certain phenolics.

CONCLUSIONS

A slightly better ethanol yield resulted from solution 2. It contained more glucose than solution 1. In solution 1, 75 percent of the sugar content was glucose; and 70 percent of the sugar content of solution 2 was glucose. Saccharomyces cerevisiae performed in a superior manner to S. uvarum in full-strength hydrolyzates, and the coculture was superior to the S. uvarum. Ethanol conversion efficiencies in diluted solutions averaged 0.45 g ethanol per g of glucose consumed.

Definite benefits for bioconversion could be seen from overneutralization of undiluted sugar solutions 1 and 2. Furfural and HMF were diminished by both overneutralization and raising the pH to 5.5 with CaO. A definite correlation can be made between disappearance of HMF and furfural, mediated by the yeasts, and appearance of ethanol. An improvement in the removal of HMF and furfural before fermentation or conditions of hydrolysis which minimize their production can have a positive effect on productivity and yield of ethanol from the sugar solutions.

The coculture was not beneficial in improving ethanol yield through ethanol production from xylose. Other strategies should be sought for

utilization of xylose which made up 19 percent of the sugars in solution 1 and 23 percent of the sugars in solution 2.

The greatest ethanol concentration achieved in the experiment was 8.9 g/L from 17.6 g/L glucose in 3 days. This represents theoretical conversion of all the glucose to ethanol. This conversion and productivity point out the need for improved productivity and for testing of sugar solutions of even greater glucose concentration.

Experimental Studies of the Progressing Batch Reactor

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ABSTRACT

A series of single percolation reactor experiments has been completed to provide a baseline for the multi-reactor experiments to follow. When operating in the liquid downflow configuration, yields of glucose ranged from 45 to 61 percent, and product concentrations of glucose from 6.4 to 9.8 g/l. When results from experiments using nominal chip sizes of 1/2 (1.27cm), 1/4 (.635cm), and 1/8 (.318cm) inches were compared, the best results were obtained with the 1/4 inch chips. The variability of results using 1/4 inch chips (not seen with 1/2 inch chips) led to the conclusion that reactor hydrodynamics plays a significant role when using nominal chip sizes smaller than 1/2 inch. The results from liquid upflow experiments were significantly inferior to those from the downflow experiments.